## PLANT GROWTH AND WATER USE AS AFFECTED BY ELEVATED $CO_2$ AND OTHER ENVIRONMENTAL VARIABLES

### CONTENTS

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### MISSION

To predict the effects of elevated $CO_2$ and climate change on the photosynthesis, growth, yield, and water use of crops under optimal and limiting levels of water and fertility.

# THE FREE-AIR $CO_2$ ENRICHMENT (FACE) PROJECT: PROGRESS AND PLANS

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**PROBLEM:** The $CO_2$ concentration of the atmosphere is increasing and is expected to double sometime during this century. Climate modelers have predicted that the increase in $CO_2$ will cause the earth to warm and precipitation patterns to be altered. This project seeks to determine the effects of such an increase in $CO_2$ and any concomitant climate change on the future productivity, physiology, and water use of crops.

**APPROACH:** Numerous $CO_2$ enrichment studies in greenhouses and growth chambers have suggested that growth of most plants should increase about 30% on the average with a projected doubling of the atmospheric $CO_2$ concentration. However, the applicability of such work to the growth of plants outdoors under less ideal conditions has been seriously questioned. The only approach that can produce an environment today as representative as possible of future fields is the free-air $CO_2$ enrichment (FACE) approach. Therefore, the FACE Project was initiated; and three experiments were conducted on cotton from 1989-1991(Hendrey, 1993; Dugas and Pinter, 1994). Then, from December 1992 through May 1994, two FACE experiments were conducted on wheat at ample and limiting levels of water supply, with about 50 scientists from 25 different research organizations in eight countries participating. Another two FACE wheat experiments were conducted on wheat from December 1995 through May 1997 at ample and limiting supplies of soil nitrogen. Over 50 papers have been published (e.g., Kimball et al., 1995, 1999; Pinter et al., 1996) or are in press from these wheat experiments, and more are being prepared.

Much of the $CO_2$ enrichment research that has been conducted in the past has been with C3 plants and relatively little with C4 crops such as corn, sugarcane, or sorghum. The neglect of C4s was because their photosynthetic process was known to respond relatively less to elevated $CO_2$. However, their stomata do partially close in elevated $CO_2$, thereby suggesting the possibility of some water conservation. Therefore, with grants (one to USWCL and one to The University of Arizona) from the NASA/NSF/DOE/USDA/EPA (TECO III) Program, we conducted two more FACE experiments on sorghum during the summer-fall growing seasons of 1998 and 1999. Our hypothesis was that there would be only a small enhancement of growth due to the FACE treatment when the plants have ample water; but under water-stressed conditions, there would be a substantial growth enhancement resulting from the water conservation due to the partial stomatal closure.

Similar to the previous FACE experiments, measurements with the sorghum included leaf area, plant height, above-ground biomass, morphological development, canopy temperature, reflectance, chlorophyll, light-use efficiency, energy balance, evapotranspiration, soil and plant elemental analyses, soil water content, photosynthesis, stomatal conductance, video observations of roots from minirhizotron tubes, soil $CO_2$ and $N_2O$ fluxes, and changes in soil C storage from soil and plant C isotopes. Some soil cores for roots also have been obtained. As before, all of the data will be assembled in a standard format for validation of plant growth models.

**FINDINGS:** Grain samples from the final FACE wheat harvests were subjected to a battery of nutritional and bread-making quality tests, as described in more detail by Kimball et al. (2001). The water stress treatment improved quality slightly with grain protein concentrations increasing relatively about 2% and bread loaf volumes about 3%. In contrast, a low soil nitrogen supply decreased quality drastically with protein decreasing about 36% and loaf volume about 26%. At ample water, elevated $CO_2$ from FACE decreased quality somewhat with protein decreasing 5% (relatively) in the irrigation experiments; but in the nitrogen experiments at ample N (which had a higher level of nitrogen than in the irrigation experiments), there was no effect of $CO_2$ on grain quality. Loaf volume was similarly decreased 2% by elevated $CO_2$ in the irrigation experiments and not affected at high nitrogen in the nitrogen experiments. Elevated $CO_2$ tended to make the deleterious effects of low nitrogen worse, with for example, protein decreasing 33% at ambient $CO_2$ and 39% under FACE. Loaf volume similarly decreased 22% at ambient and 29% under FACE.

As reported in more detail by Ottman et al. (2001), stover yield from the FACE sorghum experiments responded slightly to $CO_2$; and over the two seasons and over the Wet (ample) and Dry (water stress) irrigation treatments, it averaged 848 g $m^{-2}$ for the control and 928 g $m^{-2}$ for FACE (+9 %). In the Dry plots, grain yield increased due to elevated $CO_2$ from 472 to 553 g $m^{-2}$ (+17%) in 1998 and 106 to 142 g $m^{-2}$ (+34%) in 1999. In the Wet plots, however, grain yield was not influenced by elevated $CO_2$ in 1998, but decreased due to elevated $CO_2$ from 476 to 424 g $m^{-2}$ in 1999 (-12%). Sorghum phenological development was not affected in a consistent manner by elevated $CO_2$. Elevated $CO_2$ had the general effect of slowing growth in the Dry plots and accelerating growth in the Wet plots during the vegetative stages, but causing the reverse after anthesis during grain fill. Leaf senescence was accelerated in the Wet plots and may have been partially responsible for the lack of grain yield response to $CO_2$ with ample water. In water-stressed sorghum, stomatal closure due to elevated $CO_2$ had a negative effect on growth early and only later in the growth cycle were the positive benefits of soil water conservation realized.

**INTERPRETATION:** The FACE wheat grain quality data suggest that future elevated $CO_2$ concentrations will exacerbate the deleterious effects of low soil nitrogen on grain quality; but with ample fertilizer nitrogen, the effects will be minor.

The FACE sorghum data suggest that under conditions with ample water, the higher future atmospheric $CO_2$ concentrations may cause a slight decrease in sorghum yield due to an accelerated grain-filling period. On the other hand, under water-stress conditions, which are typical of much of the rain-fed areas where sorghum is grown in the U.S. and in Africa and other developing countries, the future higher levels of $CO_2$ are likely to increase productivity by 15% or more.

**FUTURE PLANS:** Analyses and reporting of the results from the FACE wheat and especially from the sorghum experiments will continue. Consensus from the participating investigators is that a FACE alfalfa experiment should be conducted next. Specific reasons to focus attention on alfalfa as an experimental crop are as follows: (1) Being deep-rooted, alfalfa could potentially sequester carbon at greater depths below the plow layer where the carbon may be stored for much longer periods than is possible with many other plants. (2) Because alfalfa is a perennial crop that grows the year around with about 8 cuttings per year in our climate, growth observations can be obtained over a very wide range of temperatures and, therefore, the interaction between elevated $CO_2$ and temperature can be studied. (3) Because alfalfa is a legume, the effects of elevated $CO_2$ on nitrogen fixation can be examined as can the importance of nitrogen for C sequestration. (4) Alfalfa is an important crop in the U.S. (4th in acreage behind wheat, corn, and soybeans), and it grows well in Arizona. Unfortunately, funding is not currently available to conduct such an experiment; a proposal has been submitted NASA to obtain such funding.

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**FREE-AIR CARBON DIOXIDE ENRICHMENT (FACE): EFFECTS ON SORGHUM EVAPOTRANSPIRATION IN WELL-WATERED AND WATER-STRESSED IRRIGATION TREATMENTS**

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**PROBLEM:** The 1996 Intergovernmental Panel on Climate Change (IPCC) projects that, if 1994 $CO_2$ emission levels are sustained, the global atmospheric $CO_2$ concentration will reach 500 µmol $mol^{-1}$ by the year 2100. Such an increase in $CO_2$ concentration is likely to decrease plant evapotranspiration (ET) and increase plant water-use efficiency (WUE).

**APPROACH:** Eight 490 $m^2$ rings were subjected to two levels of $CO_2$ (F = FACE, C = Control) and two levels of soil water supply (W = Wet, D = Dry) as described in detail by Ottman et. al. (2001).

Soil water content measurements: Volumetric soil moisture content was determined with the neutron probe (Hydroprobe Model 503 DR, Campbell Pacific Co., Martinez CA). The calibration equation used was: $\theta = 0.015 + 0.156 * (\text{count} / \text{standard count})$ where $\theta$ = volumetric soil water content ($\theta$: $m^3$ $H_2O$ / $m^3$ soil). Measurements were taken at 0.3 m intervals to either 1.8 m or 3.0 m depths during the 1998 and 1999 seasons, respectively.

Active root depth was determined by estimating the expected water extraction front from 0-1.76 m (Robertson et al., 1993). ET was calculated only for the zone of soil containing active roots. ET was calculated during drying periods by using a soil water balance equation (Jensen et al*.,* 1990).

**FINDINGS:** Temporal changes in soil water content: During 1998, volumetric soil water content measurements (Fig. 1a) showed two distinct dry-down periods for the Dry plots - the first from day of year (DOY) 228-254 and the second from DOY 275-330. We observed three dry-down periods in the Dry treatment during 1999: DOY 198-218, DOY 228-258, and DOY 268-290. During 1998, periods of plant stress in Dry treatments, as inferred from a 30% drop in soil moisture below field capacity, occurred during DOY 247-253 and DOY 290-325, whereas they occurred from DOY 202-218, DOY 244-258, and after DOY 280 until maturity during 1999 (Fig. 1b).

Evapotranspiration: During the first year (1998), major differences in cumulative ET between Wet and Dry treatments were apparent by DOY 273 (Fig. 2a). Aside from a divergence during DOY 260-290, when plants were undergoing reproductive growth, FD and CD treatments showed similar patterns in cumulative ET. However, significant differences between $CO_2$ treatments were evident in Wet plots beginning on DOY 260. FW plots evapotranspired 60 mm (±117 mm) or 11% less than CW over the entire growing season. Seasonal ET in the Dry treatment revealed FD evapotranspired 1 mm (±22 mm) or 0% less than CD. During 1999, differences in cumulative ET between Wet and Dry treatments began at DOY 220, becoming more pronounced after DOY 240 (Figure 2b). ET differences between $CO_2$ levels were evident within the Wet irrigation treatment from DOY 230 through the end of the season when FW had consumed 58 mm (±34 mm) or 9% less water than CW plants. Seasonal ET in the Dry treatment revealed FD evapotranspired 25 mm (±14 mm) or 6% less than CD.

**Figure 1:** Mean volumetric soil water contents (from 0.0 to 1.8 m depth) in the Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW), and FACE-Wet (FW) plots for the 1998 (1) and 1999 (b) sorghum growing seasons. Bars denote standard errors. Each mean is the average of four replicates. A indicates when only Wet plots were irrigated. B indicates when both Wet and Dry plots were irrigated. NS, *, **, *** = Not significant at P > 0.25 and significant at P < 0.25, 0.15, 0.05, respectively.

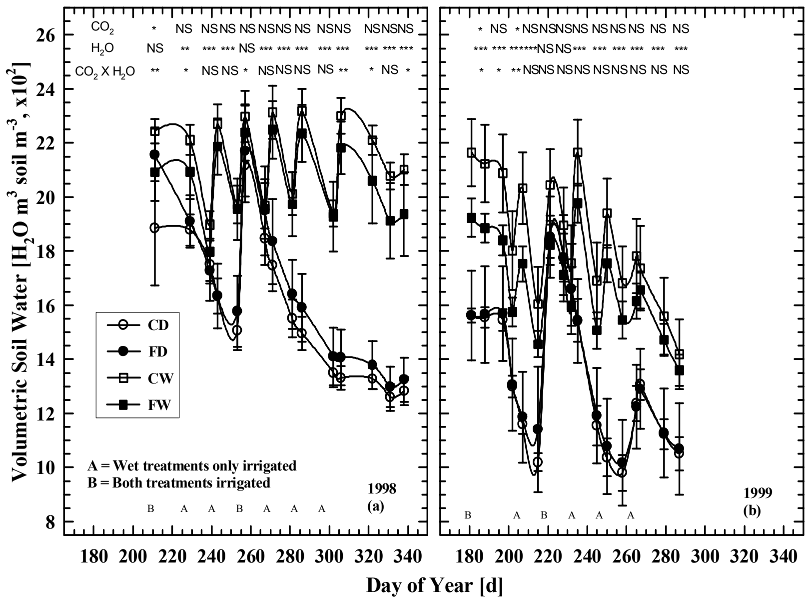
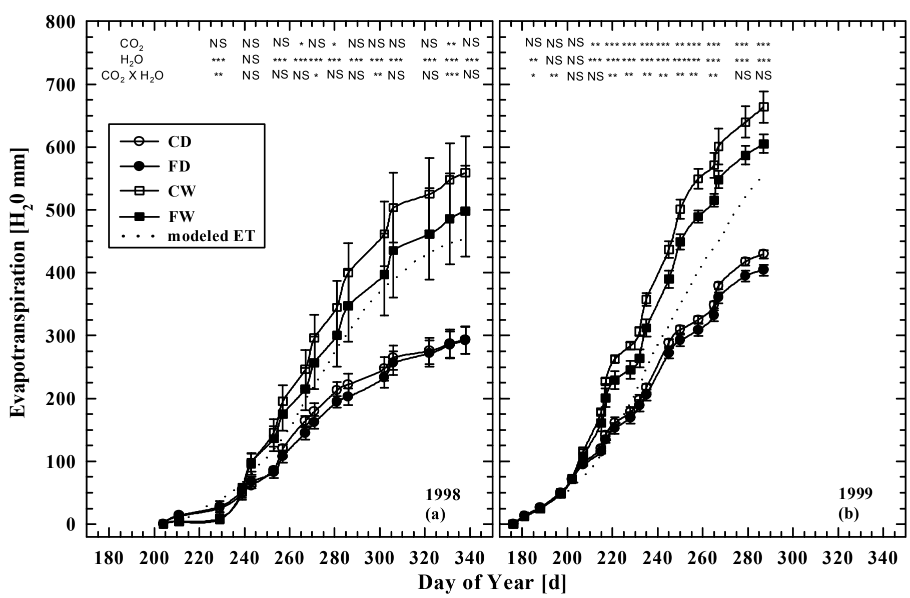



**Figure 2:**Mean cumulative evapotranspiration (ET) for the Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW), and FACE0-Wet (FW) plots in 1998 (a) and 1999 (b) sorghum growing seasons. Bars denote standard errors. Each mean is the average of four replications. The dotted line represents modeled ET from the Arizona Meteorological Network AZMET (Brown, 1987) adjusted for sorghum. NS, *, **, *** = Not significant at P > 0.25 and significant at P < 0.25, 0.15, 0.05, respectively.



Water-use Efficiency: Grain yield and total above-ground biomass were measured in 1998 and 1999 by Ottman *et al.* (2001). Water-use efficiency based on grain yield (WUE-G) was calculated as the ratio of grain yield per square meter, per mm of ET (Table 1). Water-use efficiency based on total biomass (WUE-B) was calculated as the ratio of biomass per square meter, per mm of ET (Table 2). Based on the two year average, the WUE-G for FD was 0.18 (±0.21) $g/m^2/mm$ or 19% greater than CD, and FW was 0.08 (±0.1) $g/m^2/mm$ or 9% greater than CW; WUE-B for FD was 0.5 (±0.49) $g/m^2/mm$ or 17% greater than CD, and FW was 0.42 (±0.33) $g/m^2/mm$ or 16% greater than FW. This suggests an increasing WUE due to $CO_2$ enrichment with increasing water stress. Additionally, $CO_2$ enrichment caused a larger relative increase in grain yield than in total biomass. Such yield increases in grain and total biomass are likely without additional use of water resources at higher than ambient $CO_2$ concentrations.

**Table 1:** Sorghum grain yield (Ottman et al. 2001), ET, WUE-G, and the percent difference in WUE-G based on yield and due to FACE enrichment.

| | 1998 | | | FACE | 1999 | | | FACE |
|---|---|---|---|---|---|---|---|---|
| | Yield ($g/m^2$) | ET (mm) | WUE-G ($g/m^2/mm$) | WUE-G % diff. | Yield ($g/m^2$) | ET (mm) | WUE-G ($g/m^2/mm$) | WUE-G % diff. |
| FACE-Dry | 553 (±30) | 292 (±21) | 1.93 (±0.24) | 15% (±24%) | 142 (±33) | 404 (±9) | .36 (±.09) | 45% (±26%) |
| Control-Dry | 472 (±58) | 293 (±22) | 1.68 (±0.32) | | 106 (±18) | 429 (±6) | .25 (±.04) | |
| FACE-Wet | 677 (±22) | 498 (±72) | 1.43 (±0.24) | 15% (±21%) | 424 (±21) | 605 (±15) | .70 (±.05) | -3% (±6%) |
| Control-Wet | 670 (±11) | 559 (±58) | 1.24 (±0.15) | | 475 (±13) | 664 (±25) | .72 (±.05) | |

**Table 2:** Sorghum biomass (Ottman et al. 2001), ET, WUE-B, and the percent difference in WUE-B based on biomass and due to FACE enrichment.

| | 1998 | | | FACE | 1999 | | | FACE |
|---|---|---|---|---|---|---|---|---|
| | Yield ($g/m^2$) | ET (mm) | WUE-B ($g/m^2/mm$) | WUE-B % diff. | Yield ($g/m^2$) | ET (mm) | WUE-B ($g/m^2/mm$) | WUE-B % diff. |
| FACE-Dry | 1332 (±80) | 292 (±21) | 4.64 (±0.61) | 12% (±17%) | 970 (±69) | 404 (±9) | 2.41 (±0.22) | 26% (±15%) |
| Control-Dry | 1176 (±73) | 293 (±22) | 4.13 (±0.55) | | 822 (±58) | 429 (±6) | 1.91 (±0.16) | |
| FACE-Wet | 1658 (±43) | 498 (±72) | 3.51 (±0.57) | 22% (±23%) | 1551 (±54) | 605 (±15) | 2.56 (±0.15) | 8% (±3%) |
| Control-Wet | 1554 (±5) | 559 (±58) | 2.87 (±0.30) | | 1566 (±17) | 664 (±25) | 2.37 (±0.11) | |

Increased plant water stress due to increased water demand coupled with decreased total applied water can explain the large decrease in yield, biomass, and WUE in 1999 relative to 1998.

Elevated $CO_2$ increased WUE-G by 0.13 (±0.16) $g/m^2/mm$ or 14% and WUE-B by 0.46 (±0.41) $g/m^2/mm$ or 16%. $CO_2$ enrichment caused partial stomatal closure, reduced stomatal conductance, and decreased transpiration per unit of leaf area in both Wet and Dry plots (Wall et al., 2001). Sorghum did not exhibit an increased leaf area in Wet plots (Ottman et al., 2001) so there was conservation of water (Figure 2a,b). In the Dry plots, $CO_2$-enriched plants had reduced stomatal conductance (Wall et al., 2001), which conserved water and enabled them to grow further into a drying cycle. Cumulative evapotranspiration of FD and CD plants were similar (Figure 2a,b). FD plants grew more (Ottman et al., 2001) and had a greater WUE than CD plants. Therefore, we accept the hypothesis that elevated $CO_2$ will cause sorghum to decrease ET under wet conditions and to increase WUE under both wet and dry conditions.

**INTERPERTATION:** Our data show that future water requirements for irrigated sorghum should decrease slightly, provided global warming is minimal. Under rain-fed conditions, where sorghum is more likely to experience water stress, elevated $CO_2$ will likely cause a productivity increase in total biomass and specifically grain yield. Moreover, under ample-water and, especially, water-limited conditions, increases in $CO_2$ are likely to cause WUE to increase substantially.

**FUTURE PLANS:** We plan to run ANOVA analysis of theta by depth using SAS. We will make ET and WUE measurements on alfalfa (Kimball et al, this volume).

**COOPERATORS:** See Kimball et al. (this volume).

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# ENERGY BALANCE AND EVAPOTRANSPIRATION OF SORGHUM: EFFECTS OF FREE-AIR $CO_2$ ENRICHMENT (FACE) AND SOIL WATER SUPPLY

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**PROBLEM:** The $CO_2$ concentration of the atmosphere is increasing and is expected to double sometime during this century. Climate modelers have predicted that the increase in $CO_2$ will cause the earth to warm and precipitation patterns to be altered. Such increases in $CO_2$ and possible climate change could affect the hydrologic cycle and future water resources. One component of the hydrologic cycle that could be affected is evapotranspiration (ET), which could be altered because of the direct effects of $CO_2$ on stomatal conductance and on plant growth. Therefore, one important objective of the Free-Air $CO_2$ Enrichment (FACE) Project (Kimball et al. this volume) is to evaluate the effects of elevated $CO_2$ on the ET of sorghum and other crops.

**APPROACH:** We conducted two FACE experiments on sorghum from mid-July to mid-December 1998 and again from mid-June to the end of October 1999 (Kimball et al., this volume).

Briefly, the FACE apparatus consists of the following: Four toroidal plenum rings of 25 m diameter constructed from 12" irrigation pipe were placed in a sorghum field at Maricopa, Arizona, shortly after planting. The rings had 2.5-m-high vertical pipes with individual valves spaced every 2 m around the periphery. Air enriched with $CO_2$ was blown into the rings, and it exited through holes at various elevations in the vertical pipes. Wind direction and speed were measured adjacent to each FACE ring, and $CO_2$ concentration was measured at the center of each. A computer control system used wind direction information to turn on only those vertical pipes upwind of the plots so that the $CO_2$-enriched air flowed across the plots no matter which way the wind blew. The system used the wind speed and $CO_2$ concentration information to adjust the $CO_2$ flow rates to maintain desired $CO_2$ concentrations at the centers of the rings. The FACE $CO_2$ concentration was elevated by 200 ppm $CO_2$ above ambient (about 360 ppm in daytime) 24 hr/day all season long. Four matching Control rings with blowers to provide air flow but no added $CO_2$ were also installed in the field. Some additional measurements were made in mid-field areas between the FACE and Control plots where neither $CO_2$ nor air flow were altered.

In addition to the $CO_2$ treatments, varying soil water supply was also a factor. Using a split-plot design, the main circular $CO_2$ plots were divided into semicircular halves, with each half receiving an ample irrigation regime (Wet) or else receiving a water-stress treatment (Dry). Using flood irrigation, the Wet plots were irrigated on roughly a two-week schedule, whereas the Dry plots were irrigated only twice (shortly after planting and at mid-season).

The determination of the effects of elevated $CO_2$ on $\boldsymbol{ET}$ by traditional chambers is fraught with uncertainty because the chamber walls that constrain the $CO_2$ also affect the wind flow and the exchange of water vapor. Therefore, as done previously in the FACE cotton and wheat experiments (Kimball et al., 1994, 1995, 1999), a residual energy balance approach was adopted whereby $\boldsymbol{ET}$ was calculated as the difference between net radiation, $\boldsymbol{R}_n$, soil surface heat flux, $\boldsymbol{G}_0$, and sensible heat flux, $\boldsymbol{H}$:

$$\lambda ET = R_n - G_0 - H$$

$R_n$ was measured with net radiometers and $G_0$ with soil heat flux plates. $H$ was determined by measuring the temperature difference between the crop surface and the air and dividing the temperature difference by an aerodynamic resistance calculated from a measurement of wind speed. Air temperatures were measured with aspirated psychrometers, and crop surface temperatures were measured with infrared thermometers (IRTs) mounted above each plot. Fifteen-minute averages were recorded on a datalogging system. The net radiometers and IRTs were switched weekly between the FACE and Control plots.

The instruments were calibrated carefully before the start of the experiments and again between the experiments in early 1999. During 2000, final calibrations were performed, including some comparisons among instruments from various manufacturers to use as reference standards. Analysis of the data from the standards comparisons is completed, and now analysis of the field data themselves is progressing.

**FINDINGS:** The micrometeorological data have not yet been analyzed, and so no report of the effects of the FACE treatment on the energy-balance-determined **ET** of sorghum can be made. In the prior FACE cotton experiment, the cotton had a large growth response (40% increase) to the elevated $CO_2$, but no effect on **ET** was detectable (Kimball et al., 1994). In contrast, with wheat which had a modest growth response (about 20%), the FACE treatment decreased **ET** by an average 6.7% (± 1.2%) for the four seasons under Wet, high-nitrogen conditions. Under low nitrogen and ample nitrogen, the reduction in **ET** was 19.5% (Kimball et al., 1999).

The sorghum growth response to elevated $CO_2$ amounted to about +9% averaged over two seasons and over the Wet and Dry irrigation treatments (Ottman et al., 2001; Kimball et al., this volume); so, based on the growth and energy balance comparisons from the past cotton and wheat experiments, we hypothesize that there was a slight reduction in sorghum **ET**. However, another basis for this hypothesis is that such a slight reduction was deduced from an analysis of the soil water balance by Conley et al. (this volume). They found that **ET** was reduced about 11% in Wet plots due to the FACE treatment, as averaged over the two growing seasons; whereas in the Dry plots, it was increased an average 3%.

**INTERPRETATION:** It appears from the prior FACE cotton experiments that cotton irrigation requirements will not change, whereas for wheat they may be somewhat lower in the future high-$CO_2$ world (provided that any global warming is small). We hypothesize that the sorghum irrigation requirements will also be reduced slightly, but we can make no definitive statement about them yet.

**FUTURE PLANS:** We plan to complete the analysis of the micrometeorological data from the two FACE sorghum experiments and write the corresponding manuscript.

**COOPERATORS:** See Kimball et al., "The Free-Air $CO_2$ Enrichment (FACE) Project: Progress and Plans" (this volume).

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# RELATIONSHIPS OF A-CI PARAMETERS AND ENZYME ACTIVITIES IN SORGHUM EXPOSED TO FREE-AIR $CO_2$ ENRICHMENT (FACE)

N. R. Adam and G.W. Wall, Plant Physiologists; B.A. Kimball, Supervisory Soil Scientist; P.J. Pinter Jr., Research Biologist; and R.L. LaMorte, Civil Engineer

**BACKGROUND:** With the expected doubling of atmospheric levels of $CO_2$ sometime in the 21st century, it is important to understand how this change will affect our way of life and, more generally, how it will affect plant life. The Free Atmospheric $CO_2$ Enrichment (FACE) facility at The University of Arizona Maricopa Agricultural Center is helping to determine how plants respond and acclimate to long-term exposure to elevated levels of $CO_2$ in the field.

Plants acclimate to changes in $CO_2$ concentration through changes in the amounts and activities of enzymes required to reestablish a balance within the photosynthetic apparatus. An earlier report (Adam *et al.*, 1997) presented data from a gas-exchange technique in which photosynthesis (A) was measured at a range of intracellular $CO_2$ concentrations (Ci), which provided information on changes within the photosynthetic apparatus of spring wheat, a so-called $C_3$ plant. Adam *et al.* (1998) showed that in spring wheat, the slope of the A-Ci relationship at those low values of Ci could be used to assess the changes in the activity and content of Rubisco due to growth in increased levels of $CO_2$. The work with spring wheat also indicated that, in order to fully assess the response of crop plants to elevated $CO_2$, various growth stages and canopy, a profile must be measured.

Similar experiments were conducted in 1998 and 1999 on sorghum, a warm-season crop with a different carbon-trapping mechanism, therefore, called a $C_4$ plant. The carbon-trapping enzyme of sorghum is PEPCase which, unlike Rubisco, fixes only carbon. The product of the reaction catalyzed by PEPCase is then shuttled to Rubisco and into the Calvin cycle. The relationships between the A-Ci curves and metabolic processes of $C_4$ plants are not fully understood. One objective of these experiments was to investigate the effect of growth in elevated $CO_2$ on these $C_4$ pathway enzymes in sorghum, as well as on the A-Ci relationships.

**APPROACH:** *Sorghum bicolor* (L.) Moench (cv. Dekalb 54) was planted in an open field at The University of Arizona Maricopa Agricultural Research Center, located 50 km south of Phoenix, Arizona (33.1 °N, 112.0 °W). Sorghum was planted on July 13 and 14, 1998, and again on June 14 and 15, 1999 (Kimball et al., 1999). Fifty percent emergence occurred July 30, 1998, and July 1, 1999. Following sowing, FACE apparatus was erected on site to enrich the $CO_2$ concentration of the ambient air (ca. 370 $\mu$mol mol$^{-1}$ during daytime) by 200 $\mu$mol mol$^{-1}$ above ambient. Water was applied as a split plot factor using flood irrigation such that "Wet" plots received ample water while "Dry" plots received only two irrigations and were severely stressed. All plots received 278.7 kg ha$^{-1}$ N.

For the first sampling date (the 4$^{th}$ and 5$^{th}$ leaf stages in 1998 and the 2$^{nd}$ through 5$^{th}$ leaf stages in 1999), gas exchange analyses were conducted on the uppermost fully-expanded leaf (referred to as the top leaf) and on the top minus one leaf. Thereafter, measurements were made on the top leaf and the top minus two leaf. Photosynthesis (A) rates were measured over a range of intercellular $CO_2$ (Ci) levels, generating an A-Ci curve. At the end of each curve, the leaf was frozen as quickly as possible with a liquid nitrogen-cooled clamp and stored in liquid nitrogen. Activity of Rubisco, PEPCase and PpdK were assayed from leaves collected from both years. Parameters estimated from the A-Ci curves were the initial slope, the bend (or inflection) of the curve, and the asymptote.

**FINDINGS:** The A-Ci curves in the early growth stages (i.e., 2nd and 3rd leaf stages) were found to resemble a $C_3$-type curve. However, as the plants reached the 4th and 5th leaf stage, the curves were more similar to a typical $C_4$ curve (Fig. 1a), in that the initial slope of the curve is steeper. We also found that the curves became more $C_3$-like as water stress became more severe (Fig. 1b) and became more $C_4$-like after irrigation (Fig. 1c). Step-wise regression showed that the enzyme activities measured explained 50% or less of the variation seen in the parameters of the A-Ci curves (Table 1) and that the initial activity of Rubisco was the enzyme parameter most closely related to all three portions of the curve.

**INTERPRETATION:** The initial activity of Rubisco was not expected to be related to all three portions of the curve. In addition, PEPCase was expected to be related to the initial slope of the curve. It is likely that factors other than enzyme activity are more important in determining the shape of the A-Ci curve and that the initial activity of Rubisco is responding to the factors that determine the shape of the curve. Although leaf samples from some of the measurement dates remain to be assayed, it appears that factors other than enzyme activity or concentration (for example, water status of the plant) will be more important in determining the response of sorghum plants to future climatic changes.

**FUTURE PLANS:** Biochemical assays will be conducted on both the remaining samples to determine whether these relationships change. In addition, further analysis of the effects of growth in $CO_2$ enrichment and water stress on these relationships will be conducted.

**COOPERATORS:** See Kimball et al., this volume. However, we especially wish to acknowledge the collaborative efforts of Andrew Webber of Arizona State University for helpful advice and the use of his laboratory. We also thank Jonathan Triggs and Jose Olivieri for technical assistance.

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**Figure 1**. Response of Photosynthesis (A) to Changes in Intercellular $CO_2$ concentration for the uppermost, fully-expanded leaf of sorghum in (a) pre-water stress conditions, (b) pre-irrigation and (c) post-irrigation conditions.

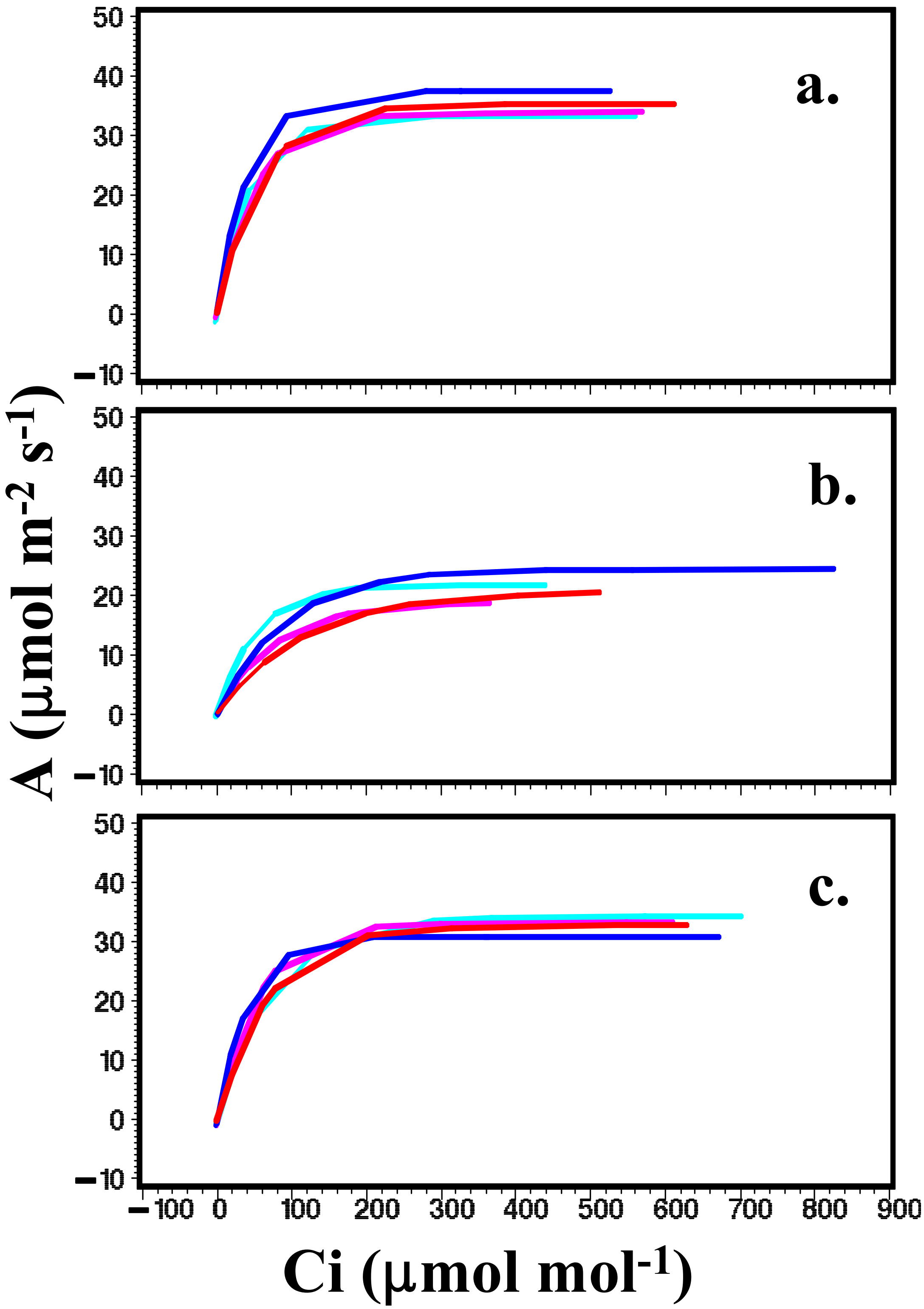

**Table 1.** Results of step-wise regression analysis of A-Ci curve parameters (Initial slope, Bend, and asymptote) with enzyme activities (Rubisco initial activity, Rubisco full activity, PEPCase optimal activity, PEPCase physiological activity, and PpdK activity).

| Parameter | Variable | Model R-Square | Pr > F |
|---|---|---|---|
| | | | |
| **Initial Slope** | Rubisco Initial Activity | 0.5137 | < 0.0001 |
| | Rubisco Full Activity | 0.5453 | 0.077 |
| | | | |
| **'Bend' of Curve** | Rubisco Initial Activity | 0.3656 | < 0.0001 |
| | PpdK Activity | 0.4337 | 0.0216 |
| | Rubisco Full Activity | 0.4779 | 0.0544 |
| | | | |
| **Asymptote** | Rubisco Initial Activity | 0.3182 | < 0.0001 |
| | PpdK Activity | 0.4153 | 0.0075 |

# Effects of Elevated $CO_2$ and Water and Nitrogen Stress on Phenology of Spring Wheat

P. J. Pinter, Jr., Research Biologist; B. A. Kimball, Supervisory Soil Scientist; G. W. Wall, Plant Physiologist; R. L. LaMorte, Civil Engineer; F. J. Adamsen, Soil Scientist; D. J. Hunsaker, Agricultural Engineer; and T.J. Brooks, Research Technician

**PROBLEM:** Terrestrial ecosystems will probably experience a significant increase in atmospheric $CO_2$ during this century. The potential effect of this change on important food and fiber crops has been the subject of extensive research by U.S. Water Conservation Laboratory (USWCL) scientists for more than a decade. In our research, we have been using Free-air Carbon dioxide Enrichment (FACE) to expose plants to supra-ambient levels of $CO_2$ because FACE minimizes the microenvironmental and climatic artifacts that are often associated with studies using open-top chambers or greenhouses. Various performance measurements have shown that the FACE facility provides very good temporal and spatial control of $CO_2$ concentrations and is a cost-effective means for large scale fumigation experiments.

Our strategies for $CO_2$ exposure and the sophistication of ambient $CO_2$ control plots have evolved over the years as FACE has been applied to different crops. Modifications have also been made as experimental objectives have changed and as more was learned about system behavior under different environmental conditions (Pinter *et al.*, 2000). When the focus of our FACE project shifted from cotton to spring wheat, a decision was made to enrich the plots on a 24h $day^{-1}$ basis. Because of the extra costs involved, the control plots used during the first two years experimentation in wheat (1992-93 and 1993-94) had dummy plastic manifolds and standpipes but were not equipped with blowers. However, with the new 24h $day^{-1}$ protocol, we began to suspect that the blowers used to introduce $CO_2$-rich air into the elevated $CO_2$ arrays was causing some slight additional microturbulence in the boundary layer above the canopy, especially during calm nights. Although subtle and difficult to measure with the mechanical cup anemometers in our micrometeorological instrumentation, the additional disturbance resulted in a slight increase in air and radiant canopy temperatures in the blower-equipped plots at night. We also suspected and later confirmed that the slightly higher temperatures were associated with differences in plant phenology and end-of-season senescence rates.

Wheat FACE experiments were carried out for another two years, through the 1995-96 and 1996-97 growing seasons. For these experiments, however, blowers were installed in the experimental control plots (hereafter called blower plots), and we did not observe differences between FACE and blower plots in nighttime air temperatures, canopy temperatures, plant phenology, or senescence. The objectives of this report are to clarify that $CO_2$ did not have a direct effect on wheat developmental rates and to discuss the overall stimulatory effect of $CO_2$ on final grain harvest from all four years of the experiment.

**APPROACH:** FACE field experiments were conducted at The University of Arizona Maricopa Agricultural Center (MAC). Spring wheat (*Triticum aestivum*, L. cv Yecora Rojo) was sown in mid-December, emerged on or about January 1, and was harvested near the end of May in each year. Irrigation was accomplished via subsurface drip tubing. A split, strip plot design incorporated two levels of $CO_2$ as the main treatment effect during all four years with levels consisting of ambient Control (~360 $\mu mol\ mol^{-1}$) and elevated FACE (a nominal 550 $\mu mol\ mol^{-1}$)

concentrations. The secondary treatment variable during the first two years ($CO_2$ by water experiments, 1992-93 and 1993-94) included two levels of irrigation: Wet (100% of consumptive requirements) or Dry (50% of Wet). The secondary treatment variable in the final two years ($CO_2$ by N experiments, 1995-96 and 1996-97) was applied nitrogen: High (~388 kg $ha^{-1}$ $yr^{-1}$) and Low (~77 kg $ha^{-1}$ $yr^{-1}$). More complete details on experimental design and treatment conditions may be found in Kimball *et al.* (1999), Hunsaker *et al.* (2000), and Pinter *et al.* (2000).

Plants were sampled at 7-10 day intervals using similar techniques all four years. Main stems were assigned growth stages according to the Zadoks scale of plant development. Dates when plants reached the midpoint of eight principal growth stages (tillering, stem elongation, booting, heading, anthesis, milk development, soft dough, and ripening) were computed via linear interpolation and then averaged for all replicates within a treatment combination. We computed the difference in chronological time required to reach a specific growth stage between plots with and without blowers (i.e., control minus FACE for 1992-93 and 1993-94) and also between plots having blowers but exposed to either ambient or elevated $CO_2$ levels (i.e., blower minus FACE for 1995-96 and 1996-97). Paired "t" tests were used to determine the statistical significance of these differences in phenology.

**FINDINGS:** Qualitative observations of plants growing in the field revealed relatively large differences in phenology (at heading, anthesis, and maturity) between control and FACE plots in the $CO_2$ by water experiments that were not evident when comparing blower and FACE plots in $CO_2$ by N experiments. Zadoks growth stage data from the periodic plant samples confirmed these observations. Development in plots with blowers was accelerated by 2 to 5 days compared to plots without blowers. The $CO_2$ treatment by itself appeared to have minimal effect on developmental rates. Paired "t" test comparisons showed the average differences in phenology between elevated $CO_2$ FACE plots and the ambient $CO_2$ control (without blowers) during 1992-93 and 1993-94 were relatively large and highly significant in both the Wet and Dry irrigation treatments (Table 1). The chronological differences in developmental rates between FACE and blower treatments during 1995-96 and 1996-97 were less than 0.5 days and only under deficit nitrogen conditions were the differences between $CO_2$ treatments statistically significant.

**Table 1**. Mean differences in the time required to reach each of 8 primary growth stages (tillering → ripening) between different Blower and $CO_2$ configurations used during the FACE Wheat experiments at Maricopa, AZ.

| | Is Blower Present ? | | | mean time | value |
|---|---|---|---|---|---|
| **Comparison**[†] | **ambient $CO_2$ treatment** | **elevated $CO_2$ treatment** | ***n***[‡] | **difference days ± 1 SE** | **from paired "*t*" test** |
| **$CO_2$ by Water Experiment (1992-93 & 1993-94)** | | | | | |
| **CW minus FW** | No | Yes | 64 | 3.2 ± 0.33 | 9.80 *** |
| **CD minus FD** | No | Yes | 64 | 2.3 ± 0.23 | 10.10 *** |
| ***$CO_2$ by Nitrogen Experiment (1995-96 & 1996-97)*** | | | | | |
| **BH minus FH** | Yes | Yes | 57 | 0.4 ± 0.31 | 1.25 NS |
| **BL minus FL** | Yes | Yes | 57 | 0.4 ± 0.18 | 2.20 * |

*, *** Significant at the 0.05 or 0.001 probability levels, respectively.
[†] Treatment abbreviations: C, Control; B, Blower; F, FACE; W, Wet irrigation; D, Dry irrigation; H, High nitrogen; L, Low nitrogen.[‡] Refers to the total number of paired observations. Only 3 replicates were available for *t* test comparisons during most of the 1996-97 experiment.

**INTERPRETATION**: One of the unique findings of the 1992-93 and 1993-94 FACE experiments was the apparent accelerating effect of elevated $CO_2$ on plant development and rates of canopy senescence (as reported by Pinter et al., 1996; and photograph in Kimball et al., 1995). A reexamination of those data in light of the slight differences in temperature we now know to exist between the treatments and the new phenology results from the $CO_2$ by N experiments have led us to conclude that the blower effect was sufficient to explain most of the apparent developmental acceleration. The subtle, blower-related rise in nighttime temperatures had cumulative effects on long-term developmental processes of the wheat plant. We now believe that elevated $CO_2$ *per se* had very little effect on the rates of plant development in well-watered and amply-fertilized spring wheat.

What effect might the blowers have had on $CO_2$ enhancement of final grain yields? We had originally reported only a 10% increase in yield for wheat exposed to $CO_2$ at 550 µmol mol$^{-1}$ and supplied with adequate water and nutrients, suggesting that plants in the Control treatment had additional opportunity to "catch up" with the sink-limited FACE plants (Pinter et al., 1996; and Kimball et al., 1995). We now believe that this 10% figure was probably an <u>underestimate</u> of the true $CO_2$ effect that might have been observed during the first 2 seasons had the controls been properly equipped with blowers and the grain filling duration of both $CO_2$ treatments been similar. In fact, during the 1995-96 and 1996-97 experiments, grain yields for adequately fertilized and well-watered wheat showed a 15% increase associated with a nominal +200 µmol mol$^{-1}$ $CO_2$ elevation. This translates into a $CO_2$ enhancement ($\beta$) factor of ~28% for a doubling of atmospheric $CO_2$ concentrations, an increase which compares favorably with the 33% average for agricultural crops reported by Kimball (1983).

There is little argument that the FACE technique approaches natural conditions more closely than open-top chambers or other means of exposing plants to elevated $CO_2$. Important advantages include an unmodified light environment, unrestricted rooting volume, and large experimental areas. Despite our results showing that the blowers cause a slight modification of crop microclimate, the advantages of FACE over open-top chambers and greenhouses still outweigh disadvantages by a considerable margin. However, these findings emphasize the importance of equipping control plots in FACE facilities with blowers and point out a disadvantage to nighttime $CO_2$ enrichment in our region.

**FUTURE PLANS:** A manuscript dealing with interactive effects of elevated $CO_2$ and water or nitrogen stress on wheat plant growth and final yields is in preparation.

**COOPERATORS:** S. Leavitt, A. Matthias, T. Thompson, and S. White, The University of Arizona, Tempe AZ; B. Roth, P. Murphree, J. Chernicky, and R. Rauschkolb (deceased), Maricopa Agricultural Center, Maricopa AZ; K. Lewin, J. Nagy, and G. Hendrey, Brookhaven National Laboratory, Uptown NY; and F. and G. Wechsung, S. Grossman-Clarke, and T. Kartschall, Potsdam Institute for Climate Research, Potsdam, Germany. We also thank R. Rokey, S. Gerszewski, R. Seay and D. Pabian for technical assistance in the field and R. Altamarano, M. Baker, C. O'Brien, H. Steinman, and K. West for processing the plant samples.

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# LONG -TERM $CO_2$ ENRICHMENT OF SOUR ORANGE TREES: EFFECTS ON PRODUCTIVITY AND CARBON SEQUESTRATION

S.B. Idso, Research Physicist; and B.A. Kimball, Supervisory Soil Scientist

**PROBLEM:** Many people believe the ongoing rise in the air's $CO_2$ content is the greatest problem ever to be faced by humanity based on the assumption that it could lead to catastrophic global warming via intensification of the planet's natural greenhouse effect. However, elevated concentrations of atmospheric $CO_2$ also provide many benefits, some of which tend to ameliorate the global warming problem. Earth's trees, for example, account for approximately two-thirds of the planet's photosynthesis, by which means they remove prodigious amounts of $CO_2$ from the air and sequester its carbon in their tissues and the soil beneath them, thereby slowing its rate of rise in the atmosphere. Consequently, we seek to determine the direct effects of atmospheric $CO_2$ enrichment on all aspects of the growth and development of trees concentrating on the long-term aspects of the phenomenon; for until someone conducts an experiment measured in *decades*, we will never know the ultimate impact of the ongoing rise in the air's $CO_2$ content on the planet's most powerful contemporary carbon sink.

**APPROACH:** In July 1987, eight 30-cm-tall sour orange tree (*Citrus aurantium* L.) seedlings were planted directly into the ground at Phoenix, Arizona. Four identically-vented, open-top, clear-plastic-wall chambers were then constructed around the young trees which were grouped in pairs. $CO_2$ enrichment – to 300 ppmv (parts per million by volume) above ambient – was begun in November 1987 in two of these chambers and, other than for brief maintenance and construction periods, has continued unabated since that time. Except for this differential $CO_2$ enrichment of the chamber air, all of the trees have been treated identically, being irrigated and fertilized as deemed appropriate for normal growth (Idso and Kimball, 1997).

As in all prior years, we continue to measure the circumferences of the trunks of the trees at the midpoint of each month; and from these data, we calculate monthly values of total trunk plus branch volume on the basis of relationships developed specifically for our trees (Idso and Kimball, 1992). Then, from wood density (dry mass per fresh volume) measurements we have made over the past several years, we calculate monthly values of the total dry weight of the trunk and branch tissue of each tree. Results for December, January, February and March – the winter period of minimal trunk expansion – are then averaged to give a mean value for the year, from which the preceding year's mean value is subtracted to yield the current year's production of trunk and branch biomass.

We likewise continue our yearly fruit measurements, counting the number of fruit to reach maturity on each tree, weighing the fruit, and calculating the total dry weight of fruit produced in each of the $CO_2$ treatments from previous determinations of fruit percent dry weight. Also from previously derived relationships (Idso and Kimball, 1992), we evaluate the number of new leaves produced each year from our trunk circumference measurements; and from bimonthly assessments of leaf dry weight similar to those of Idso et al. (1993), we calculate the total dry weight of leaves produced each year. These results, added to the trunk and branch dry weights and fruit dry weights, then give us the total aboveground dry weight production per year for all of the trees in the two $CO_2$ treatments.

When viewed in their entirety, the results continue to be encouraging. They indicate that the trees of both $CO_2$ treatments may be close to achieving a stage of maturity characterized by a near-steady-state of yearly aboveground biomass production (Fig. 1). For the last five years of the experiment, for example, the values of total aboveground biomass in the $CO_2$-enriched trees were 107, 90, 95, 116, and 89 kg/tree; while those for the ambient-treatment trees were 62, 51, 57, 61, and 55 kg/tree, producing a five-year-mean $CO_2$-enriched/ambient-treatment ratio of 1.74.

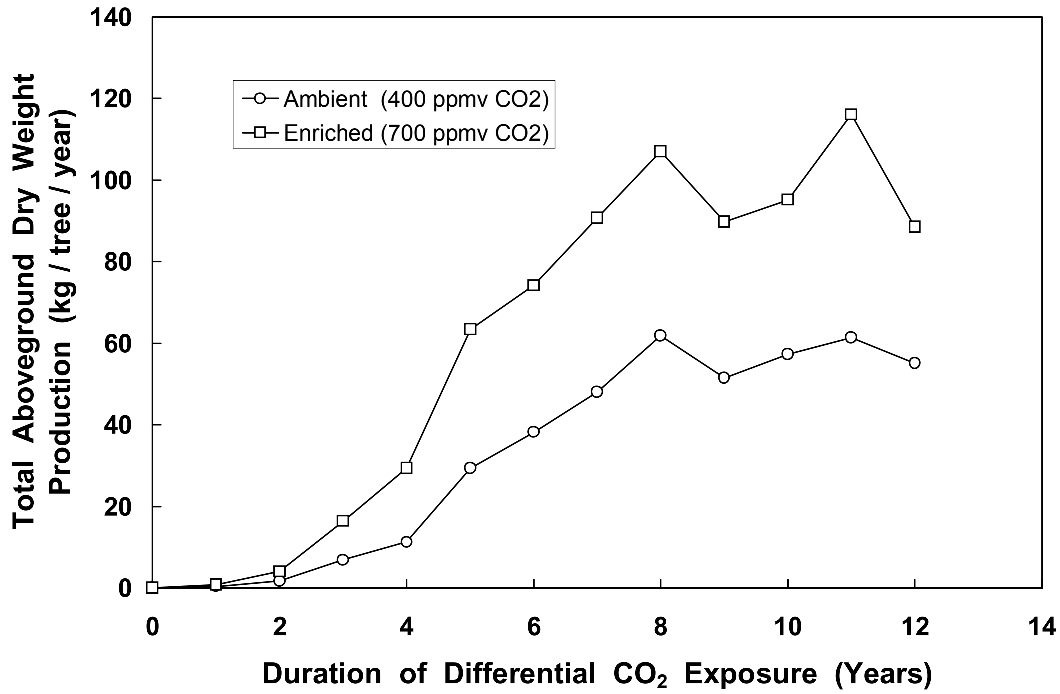


**Figure 1.** Yearly total aboveground biomass production in the ambient and $CO_2$-enriched sour orange trees as a function of time since the start of the experiment.

The fruit production component of the total aboveground productivity has been a little more erratic; nevertheless, it too appears to be approaching an asymptotic upper limit (Fig. 2). For the last five years, for example, harvested fruit biomass has been 47, 38, 38, 58, and 36 kg/tree in the $CO_2$-enriched trees; while in the ambient-treatment trees it has been 25, 13, 23, 31, and 22 kg/tree, producing a five-year-mean $CO_2$-enriched/ambient-treatment fruit production ratio of 1.87.

We have also discovered that in the spring of each year the $CO_2$-enriched trees experience an enormous growth enhancement. This initial stimulation begins immediately upon bud-burst; and three to four weeks later, the new branches of the $CO_2$-enriched trees may be four times more massive than those of the ambient-treatment trees. Furthermore, because there are more branches on the $CO_2$-enriched trees, they may have as much as six times more total new-branch biomass than the ambient-treatment trees. Shortly thereafter, however, a decline sets in and the $CO_2$-enriched/ambient-treatment new-branch biomass ratio of the trees ultimately levels out at a value commensurate with the long-term total aboveground productivity ratio of the $CO_2$-enriched and ambient-treatment trees; i.e., at a value of approximately 1.74.

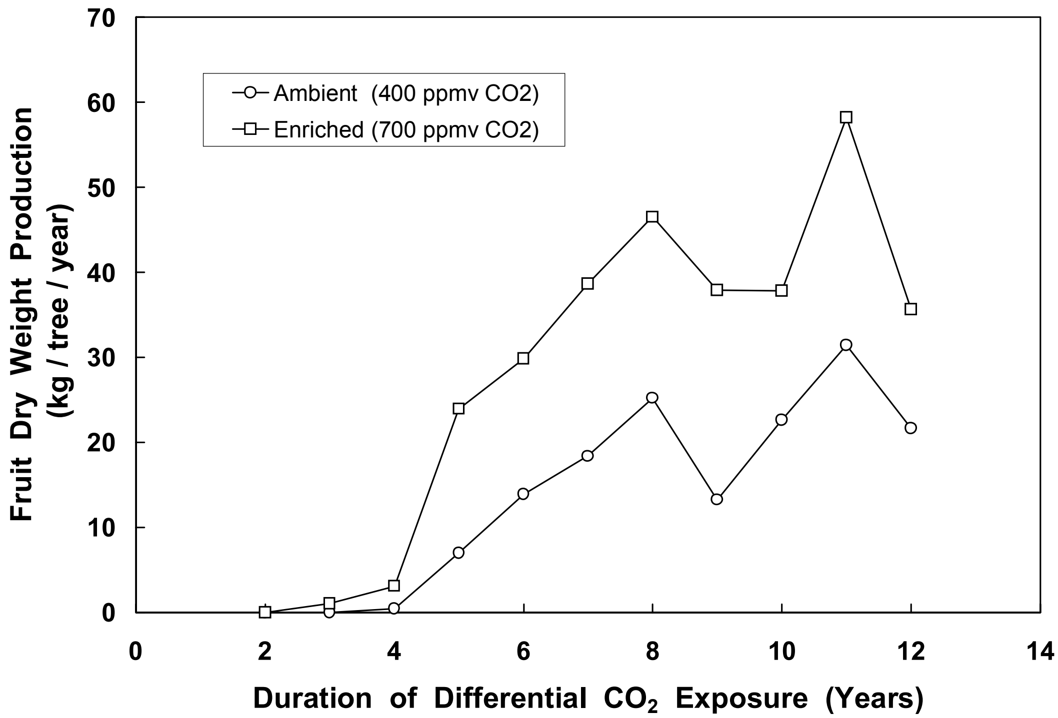


**Figure 2.** Yearly fruit dry weight production in the ambient and $CO_2$-enriched sour orange trees as a function of time since the start of the experiment.

**INTERPRETATION:** What is the ultimate fate of the $CO_2$ the people of the world yearly emit to the atmosphere? Will the trees of the planet be sufficiently stimulated by the ongoing rise in the air's $CO_2$ content to remove enough of it from the atmosphere to prevent a significant $CO_2$-induced warming of the globe? The results of our ongoing study provide important insight into these questions and may help our government craft appropriate policies to meet this global environmental challenge. In the meantime, our findings continue to demonstrate that carbon dioxide is an effective aerial fertilizer, significantly increasing the size, growth rate, and fruit production of sour orange trees exposed to more of this aerial fertilizer than is normally in the air. It is also possible that this phenomenon may be partially responsible for the progressively earlier occurrence of the spring "green up" of the Northern Hemisphere's vegetation, which has been observed over the past few decades in satellite studies of surface reflectance and in the increasingly earlier occurrence of the spring draw-down of the air's $CO_2$ content that is evident in studies of the atmosphere's seasonal $CO_2$ cycle (Idso et al., 2000).

**FUTURE PLANS:** We plan to continue the sour orange tree experiment as long as it takes to determine if the trees truly achieve steady-state yearly growth rates and if the $CO_2$-enriched trees are maintaining a growth advantage over the ambient-treatment trees that can reasonably be expected to continue indefinitely. We are also continuing our investigation of the ultra-enhanced spring branch growth phenomenon that we have observed in the $CO_2$-enriched trees, having just completed three full years of pertinent measurements. In addition, we have initiated several new research thrusts related to the central problem of carbon sequestration. In cooperation with a soil microbiologist, we are investigating the role of atmospheric $CO_2$ enrichment in stimulating the growth of soil fungi that grow in symbiotic association with the sour orange tree roots and produce a glycoprotein called glomalin, which has been proven to enhance soil aggregation and the stability of soil aggregates. And in cooperation with several scientists who are expert in various types of tree-ring analyses, we are studying cores of the sour orange tree trunks for $CO_2$-induced differences in cell size, wood density and strength properties. We are also collecting some new data sets that should shed even more light on the effects of atmospheric $CO_2$ enrichment on the trees' physiology, including weekly assessments of leaf fall and leaf concentrations of chlorophyll, starch and various sugars. Finally, we are entering upon our tenth year of fruit vitamin C measurements and our fourth year of fruit folic acid measurements.

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# ELEVATED ATMOSPHERIC $CO_2$ ALLEVIATES WATER-STRESS-INDUCED MID-AFTERNOON DEPRESSION IN WHEAT CARBON GAIN

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**PROBLEM:** Atmospheric $CO_2$ concentration is on the rise, which can affect stomatal conductance and water absorption processes in wheat (*Triticum aestivum* L.). Elevated $CO_2$ also affects internal plant water potential, and as is well known, it also affects photosynthesis. The degree to which each of these processes is affected will determine the overall productivity of wheat in the future. An imperative exists, therefore, to determine the effect that a future high $CO_2$ -world will have on dawn to dusk trends in stomatal conductance ($g_s$), total ($\Psi_W$), osmotic ($\Psi_\pi$), and turgor ($\Psi_P$) leaf potentials and net assimilation rate (A) of wheat grown under elevated $CO_2$ and adequate and limited soil-water content.

**APPROACH:** A 2-year field study on a hard red spring wheat (cv. Yecora Rojo) crop was conducted in an open field at The University of Arizona Maricopa Agricultural Center located 50 km south of Phoenix, Arizona. Seeds were sown into flat beds at 0.25-m row spacings on December 15, 1992, (130 plants $m^{-2}$) and December 7-8, 1993 (180 plants $m^{-2}$). The crops were harvested on May 25-27, 1993, and on June 1, 1994. Following sowing, a free-air $CO_2$ enrichment (FACE) apparatus was erected on site to enrich the $CO_2$ concentration of ambient air (~350 µmol $mol^{-1}$) to 550 µmol $mol^{-1}$ treatment level (main plots) for 24 h per day from 50% emergence until physiological maturity. A subsurface drip-tape irrigation system provided two soil-water content (I) treatments; 50% (Dry) and 100% (Wet) replacement of potential evapotranspiration (split-plot). Treatments combinations, therefore, consisted of Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW) and FACE-Wet (FW). There were four replications of each treatment combination.

A portable closed gas exchange system (0.25 L transparent cuvette) was used to make in situ measurements of $g_s$ and A on uppermost fully expanded sunlit leaves from dawn to dusk. Leaves were excised approximately 5 mm away from the leaf collar to measure $\Psi_W$ with a pressure chamber. Measurements of $\Psi_W$ and $\Psi_\pi$ were also made at midday with leaf thermocouple psychrometers using standard psychrometric techniques, whereas turgor pressure ($\Psi_P$) was derived ($\Psi_P = \Psi_W - \Psi_\pi$).

**FINDINGS:** FACE reduced $g_s$ by 30% at mid-morning (2.5 h prior to solar noon), 34% at midday (solar noon), and 34% at mid-afternoon (2.5 h after solar noon) (Fig. 1). In Dry compared with Wet, FACE caused less of a proportionate reduction in $g_s$ than Control by 2% at mid-morning and midday, but by 25% at mid-afternoon. Full irrigation increased $g_s$ by 66% at mid-morning and midday and 79% at mid-afternoon. Overall, $\Psi_W$ was less negative by 0.16 and 0.33 MPa at midday in FACE compared with Control, and $\Psi_W$ was less negative by 0.25 and 0.59 MPa in Wet compared with Dry during mild (1993) and severe (1994) drought stress years, respectively. Osmotic adjustment ($\Delta\Psi_\pi$) was 0.73 [$\Psi_\pi$ MPa ($\Psi_W$ MPa)$^{-1}$] for Control and 0.81 [$\Psi_\pi$ MPa ($\Psi_W$ MPa)$^{-1}$] for FACE ($P$=0.20), whereas loss of turgor ($\Delta\Psi_P$) was 0.18 [$\Psi_P$ MPa ($\Psi_W$ MPa)$^{-1}$] for Control and 0.16 [$\Psi_P$ MPa ($\Psi_T$ MPa)$^{-1}$] for FACE ($P$=0.20).

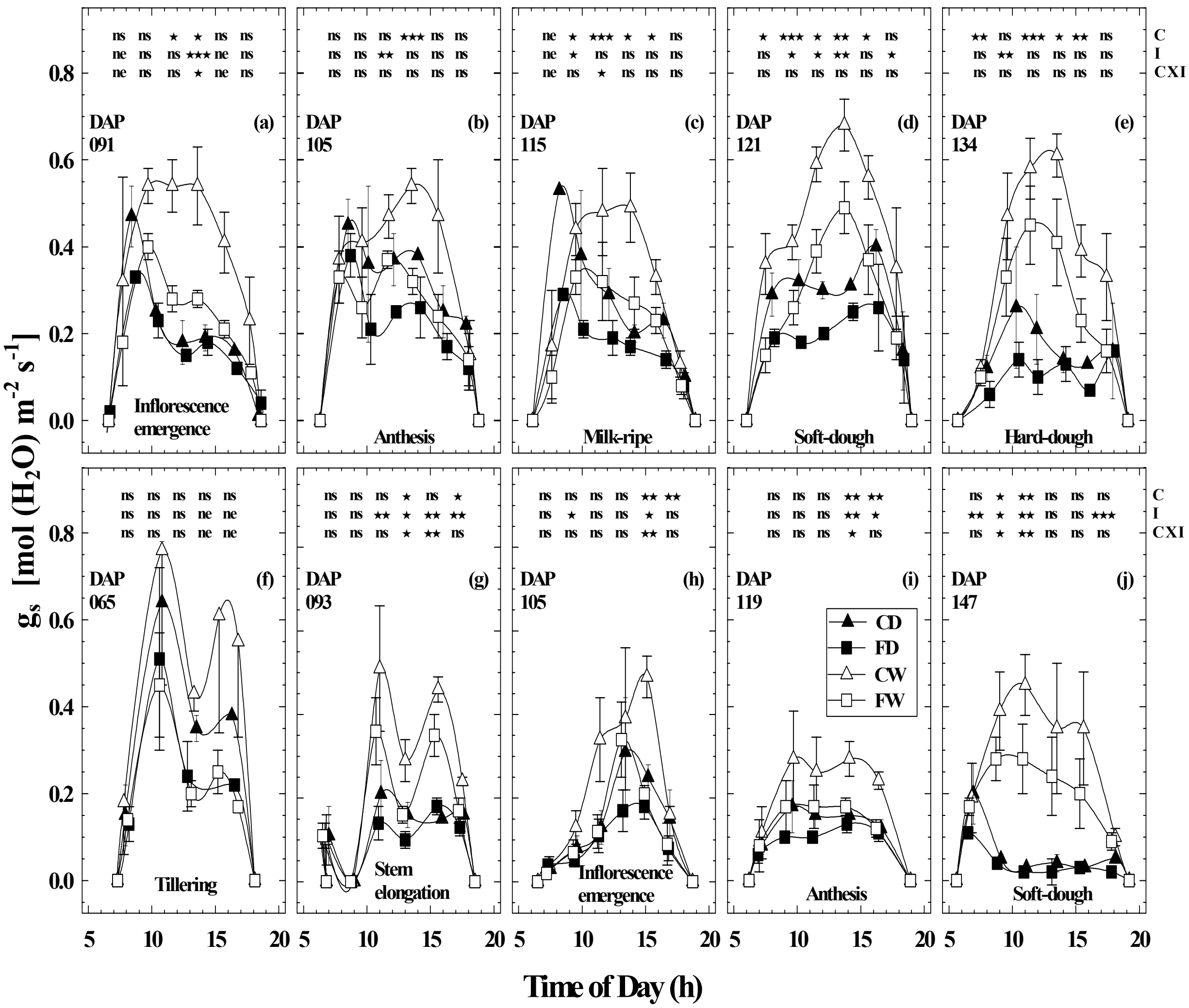


**Figure 1**. DAWN TO DUSK TRENDS IN STOMATAL CONDUCTANCE ($G_S$) OF FULLY EXPANDED SUNLIT SPRING WHEAT LEAVES FOR DAY AFTER PLANTING (DAP) AND GROWTH STAGES GIVEN FOR 5 D DURING 1993 (A-E) AND 1994 (F-J). SYMBOLS IN LEGEND REFER TO CONTROL-DRY (CD), CONTROL-WET (CW), FACE-DRY (FD), AND FACE-WET (FW) TREATMENTS. Each vertical bar is one standard error from each datum. Source of variance in ANOVA are carbon dioxide [(C): Control at 370 µmol mol$^{-1}$, and FACE at 550 µmol mol$^{-1}$], irrigation effect [(I): Dry at 50% and Wet at 100% replacement of evapotranspiration], and CxI interaction effects. Significance effects given for each growth stage above each datum as ★★★, ★★, ★, and ns for $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.10$, and not significant (ne for effect not estimated), respectively. Actual probability of a greater F-value by chance reported if $P \geq 0.10$ and $P \leq 0.25$.

Compared with Control, FACE stimulated A by 32% at mid-morning, 25% at midday,and 23% at mid-afternoon (Fig. 2). The stimulation of A by FACE was greater by 7% at mid-morning and midday and 35% at mid-afternoon under Dry than Wet. Elevated $CO_2$, therefore, alleviated water-stress-induced mid-afternoon depressions in wheat carbon gain. Full irrigation increased A by 13, 29, and 28% at mid-morning, midday, and mid-afternoon, respectively.

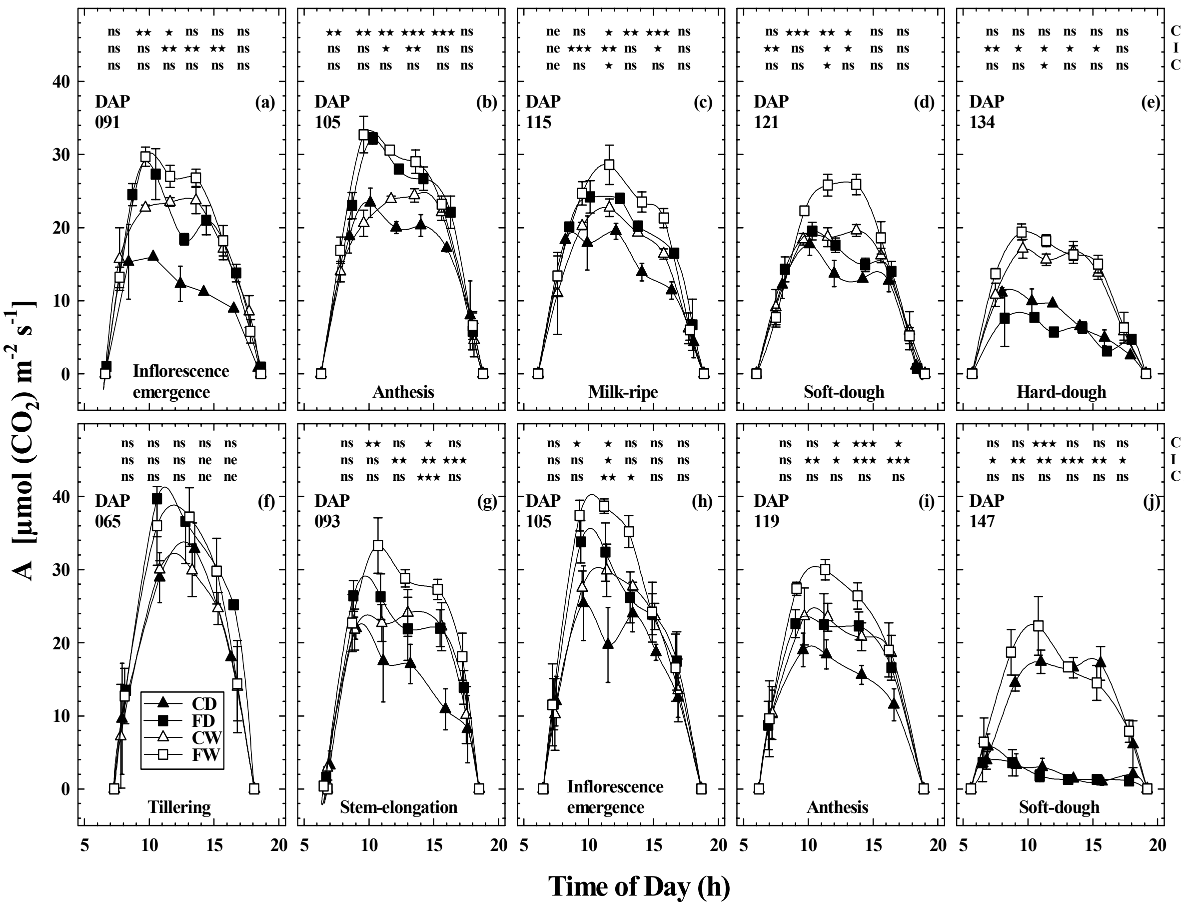


**Figure 2**. DAWN TO DUSK TRENDS IN LEAF NET ASSIMILATION RATE (A) OF EXPANDED SUNLIT SPRING WHEAT LEAVES FOR DAY AFTER PLANTING (DAP) AND GROWTH STAGES GIVEN FOR 5 D DURING 1993 (A-E) AND 1994 (F-J). SYMBOLS IN LEGEND SAME AS GIVEN IN FIG. 1. SOURCE OF VARIANCE AND RESULTS FROM ANOVA SAME AS DESCRIBED IN FIG. 1.

A hysteresis effect was observed when A vs. photosynthetic photon flux density (PPFD) was plotted from dawn until midday compared to that from midday to dusk (Fig. 3). This hysteresis effect became more pronounced as soil-water content became more depleted. Relationships between A vs. PPFD from dawn to midday and from midday to dusk were normalized ($A_n$) by dividing each observation of A within a day by the maximum value of A ($A_{max}$) for that day (usually at mid-morning or midday). Clearly, no hysteresis effect occurred under CW, a slight hysteresis effect was observed under FW, but only a modest increase in this effect was observed for FD. In contrast, a large hysteresis effect was observed for CD (Fig. 3). The smaller the hysteresis effect the greater was daily ($A'$) and seasonal accumulation ($A''$) of carbon. Compared with Control, FACE increased $A'$ and $A''$ by 25%. However, the stimulation in $A''$ by FACE over Control was 31% greater under Dry, but only 21% under Wet. This 10% difference in $A''$ between Dry and Wet can be explained by a proportionate reduction in the hysteresis effect observed for FD compared with CD.

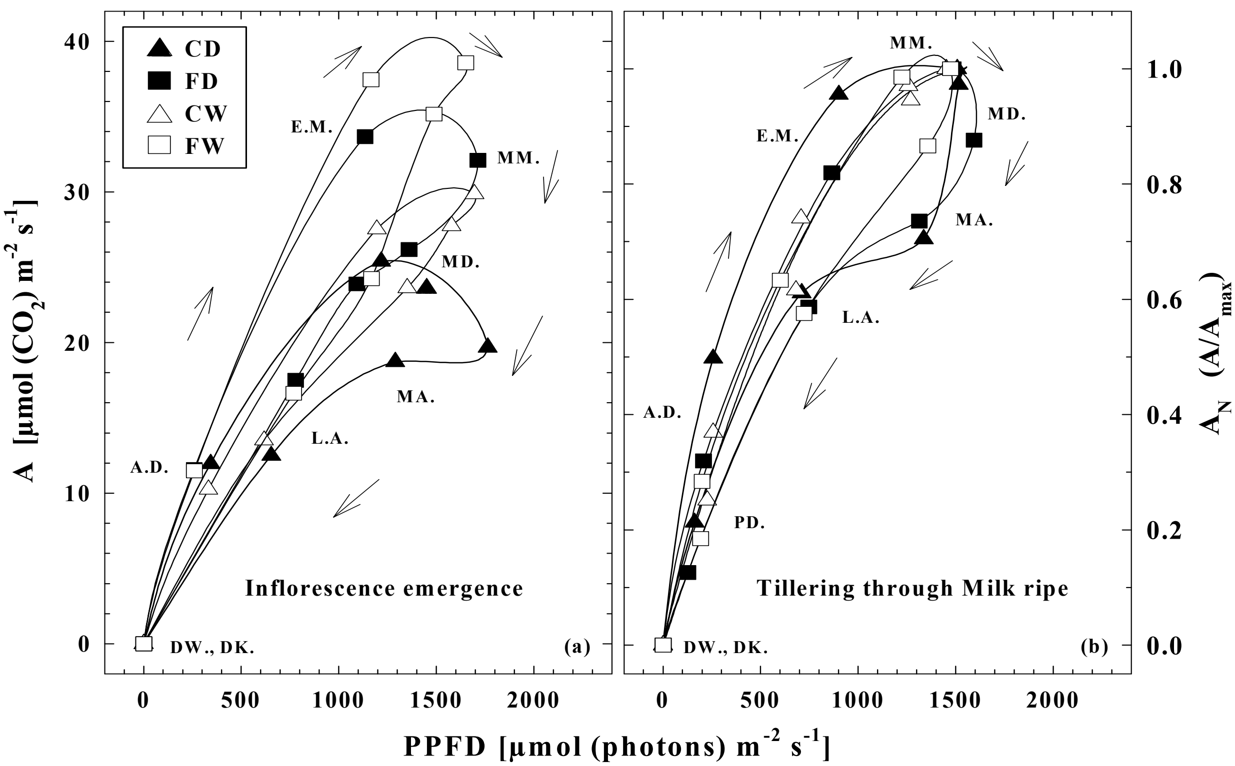


**Figure 3**: Mean leaf net assimilation rate (A) in response to diurnal course of incident photosynthetic photon flux density (PPFD) during inflorescence emergence on day after planting 105 (replotted from Fig. 2h) (a). Mean normalized leaf net assimilation rate ($A_N$), across years and growth stages except soft and hard-dough (replotted from Fig. 2a-d and f-i), in response to the diurnal course of incident photosynthetic PPFD (b). Arrows denote direction of hysteresis loop from DW-dawn, AD-after dawn, EM-early morning, MM-mid-morning, MD-midday, MA-mid-afternoon, LA-late-afternoon, PD-pre-dusk, and DK-dusk. Symbol legend same as given in Fig. 1.

**INTERPRETATION:** Despite the fact that elevated $CO_2$ directly reduced $g_s$ (Fig. 1), it indirectly increased drought avoidance (reduced evapotranspiration and increased capacity for absorption of water and nutrients by roots), and increased drought tolerance (xeromorphic adaptations and osmoregulation mechanisms). Consequently, as water stress became more severe, the mitigating effect of elevated $CO_2$ in alleviating drought actually caused a reduction in water-stress-induced stomatal limitation in wheat carbon gain, particularly at mid-afternoon. Nevertheless, these results also demonstrate that although leaves grown under elevated $CO_2$ will have less stomatal limitations, regardless of water supply, they will still experience some mid-afternoon depression in carbon gain because of non-stomatal limitations (photoinhibition). In a future high-$CO_2$ world, therefore, additional carbon uptake at mid-afternoon will increase $A'$ and $A''$. Presumably, this additional carbon supply will result in an increase in total non-structural carbohydrate pools. Hence, despite any limitations in net primary production because of water deficits, a rise in atmospheric $CO_2$ will minimize the deleterious effects of drought on physiological function and growth, thereby, expanding the range where a viable crop can be grown, especially under dryland conditions.

**FUTURE PLANS:** We will continue to analyze, interpret, summarize, and document results from previous FACE experiments on wheat and sorghum. We will also actively plan and seek funding for a FACE alfalfa (*Medicago sativa* L.) experiment (Kimball et al., this volume).

**COOPERATORS:** See report from Kimball et al., this volume.